

FIG. 1

| | | | | | | | | | | | | | |
|-----------------------------------------------------|-----|----|--|--|--|--|--|--|--|--|--|--|--|
| 1 | 5 | 10 | | | | | | | | | | | |
| ATG GCT CCA ATG ACT CAG ACT ACT TCT CTT AAG ACT TCT | | | | | | | | | | | | | |
| Met Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser | | | | | | | | | | | | | |
| 15 | 20 | | | | | | | | | | | | |
| TGG GTT AAC TGC TCT AAC ATG ATC GAT GAA ATT ATA ACA | | | | | | | | | | | | | |
| Trp Val Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr | | | | | | | | | | | | | |
| 30 | 35 | | | | | | | | | | | | |
| CAC TTA AAG CAG CCA CCT TTG CCT TTG CTG GAC TTC AAC | | | | | | | | | | | | | |
| His Leu Lys Gln Pro Pro Leu Pro Leu Leu Asp Phe Asn | | | | | | | | | | | | | |
| 40 | 45 | | | | | | | | | | | | |
| AAC CTC AAT GGG GAA GAC CAA GAC ATT CTG ATG GAA AAT | | | | | | | | | | | | | |
| Asn Leu Asn Gly Glu Asp Gln Asp Ile Leu Met Glu Asn | | | | | | | | | | | | | |
| 55 | 60 | | | | | | | | | | | | |
| AAC CTT CGA AGG CCA AAC CTG GAG GCA TTC AAC AGG GCT | | | | | | | | | | | | | |
| Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg Ala | | | | | | | | | | | | | |
| 65 | 70 | | | | | | | | | | | | |
| GTC AAG AGT TTA CAG AAT GCA TCA GCA ATT GAG AGC ATT | | | | | | | | | | | | | |
| Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile | | | | | | | | | | | | | |
| 80 | 85 | | | | | | | | | | | | |
| CTT AAA AAT CTC CTG CCA TGT CTG CCC CTG GCC ACG GCC | | | | | | | | | | | | | |
| Leu Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala | | | | | | | | | | | | | |
| 95 | 100 | | | | | | | | | | | | |
| GCA CCC ACG CGA CAT CCA ATC CAT ATC AAG GAC GGT GAC | | | | | | | | | | | | | |
| Ala Pro Thr Arg His Pro Ile His Ile Lys Asp Gly Asp | | | | | | | | | | | | | |
| 105 | 110 | | | | | | | | | | | | |
| TGG AAT GAA TTC CGT CGT AAA CTG ACC TTC TAT CTG AAA | | | | | | | | | | | | | |
| Trp Asn Glu Phe Arg Arg Lys Leu Thr Phe Tyr Leu Lys | | | | | | | | | | | | | |
| 120 | 125 | | | | | | | | | | | | |
| ACC TTG GAG AAC GCG CAG GCT CAA CAG ACC ACT CTG TCG | | | | | | | | | | | | | |
| Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr Leu Ser | | | | | | | | | | | | | |
| 130 | | | | | | | | | | | | | |
| CTA GCG ATC TTT TAA TAA | | | | | | | | | | | | | |
| Leu Ala Ile Phe END END | | | | | | | | | | | | | |

(SEQ ID NO: 144)

(SEQ ID NO: 138)

C
 I
 a
 I
 aa20 ATCGATGAATCATCACCACCTGAAGCAGCCACCGTCCCGTGTGGACTTCAACAAC
 1 -----+-----+-----+-----+-----+ 60
 IleAspGluIleIleThrHisLeuLysGlnProProLeuProLeuLeuAspPheAsnAsn -

 E
 C
 o
 R
 V
 x
 h
 o
 I
 CTCAATGGTGAAGACCAAGATATCCTGATGGAAATAACCTTCGTCTCGTCCAAACCTCGAG
 61 -----+-----+-----+-----+-----+ 120
 LeuAsnGlyGluAspGlnAspIleLeuMetGluAsnAsnLeuArgArgProAsnLeuGlu -

 P
 S
 t
 I
 N
 S
 i
 I
 GCATTCAACCGTGTCTCAAGTCTCTGCAGAAATGCAT [SEQ ID NO:145]aa70
 121 -----+-----+-----+-----+-----+ 157
 AlaPheAsnArgAlaValLysSerLeuGlnAsnAla [SEQ ID NO:146]

ClaI to NsiI Replacement Fragment

Fig - 2

N H
 C P
 O a
 I I

1 CCATGGGCTCCAATGACTCAGACTACTTCTCTTAAGACTTCTTGGGTTAACTGCTCTAACA
 -----+-----+-----+-----+-----+ 60
 GGTACCGAGGTTACTGAGTCTGATGAAGAGAATTCTGAAGAACCCAATTGACGAGATTGT

MetAlaProMetThrGlnThrThrSerLeuLysThrSerTrpValAsnCysSerAsnMet

C
 l
 a
 I

61 TGATCGATGAAATTATAACACACTTAAAGCAGCCACCTTTGCCTTTGCTGGACTTCAACA
 -----+-----+-----+-----+-----+ 120
 ACTAGCTACTTTAATATTGTGTGAATTTCGTCGGTGGAACGGAACGACCTGAAGTTGT

IleAspGluIleIleThrHisLeuLysGlnProProLeuProLeuLeuAspPheAsnAsn

121 ACCTCAATGGGGAAGACCAAGACATTCTGATGGAAAATAACCTTCGAAGGCCAAACCTGG
 -----+-----+-----+-----+-----+ 180
 TGGAGTTACCCCTTCTGGTTCTGTAAGACTACCTTTTATTGGAAGCTTCCGGTTTGGACC

LeuAsnGlyGluAspGlnAspIleLeuMetGluAsnAsnLeuArgArgProAsnLeuGlu

N
 S
 i
 I

181 AGGCATTCAACAGGGCTGTCAAGAGTTTACAGAATGCATCAGCAATTGAGAGCATTTCTTA
 -----+-----+-----+-----+-----+ 240
 TCCGTAAGTTGTCCCGACAGTTCTCAAATGTCTTACGTAGTCGTTAACTCTCGTAAGAAT

AlaPheAsnArgAlaValLysSerLeuGlnAsnAlaSerAlaIleGluSerIleLeuLys

240 AAAATCTCCTGCCATGTCTGCCCTTGGCCACGGCCGACCCACGCGACATCCAATCCATA
 -----+-----+-----+-----+-----+ 300
 TTTTAGAGGACGGTACAGACGGGGACCGGTGCCGGCGTGGGTGCGCTGTAGGTTAGGTAT

AsnLeuLeuProCysLeuProLeuAlaThrAlaAlaProThrArgHisProIleHisIle

E
C
O
R
I

301 TCAAGGACGGTGACTGGAATGAATTCGGTCGTAAACTGACCTTCTATCTGAAAACCTTGG
-----+-----+-----+-----+-----+ 360
AGTTCCTGCCACTGACCTTACTTAAGGCAGCATTGACTGGAAGATAGACTTTTGGGAACC
LysAspGlyAspTrpAsnGluPheArgArgLysLeuThrPheTyrLeuLysThrLeuGlu

N H
h i
e d
I I
I I

361 AGAACGCGCAGGCTCAACAGACCACTCTGTCGCTAGCGATCTTTAATAAGCTT
-----+-----+-----+-----+-----+ 414
TCTTGCGCGTCCGAGTTGTCTGGTGAGACAGCGATCGCTAGAAAATTATTCGAA
AsnAlaGlnAlaGlnGlnThrThrLeuSerLeuAlaIlePheEndEnd

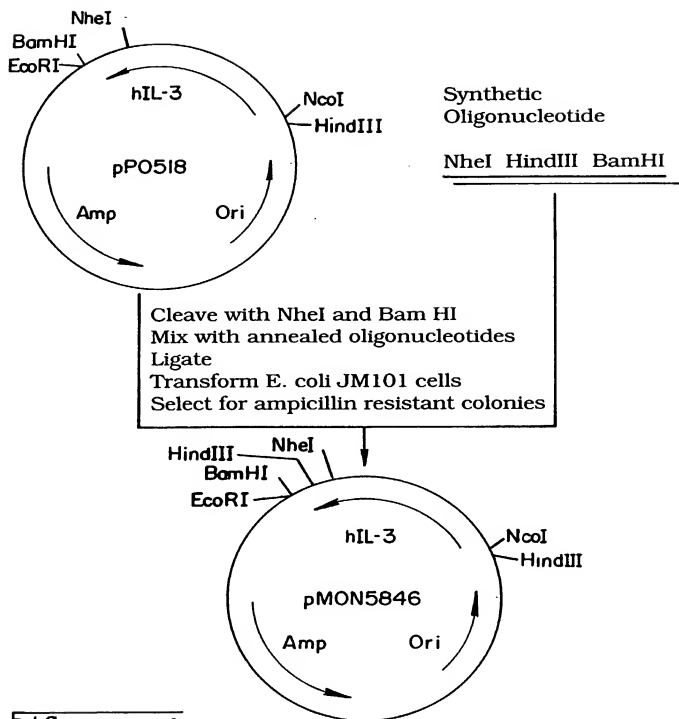


Fig- 4

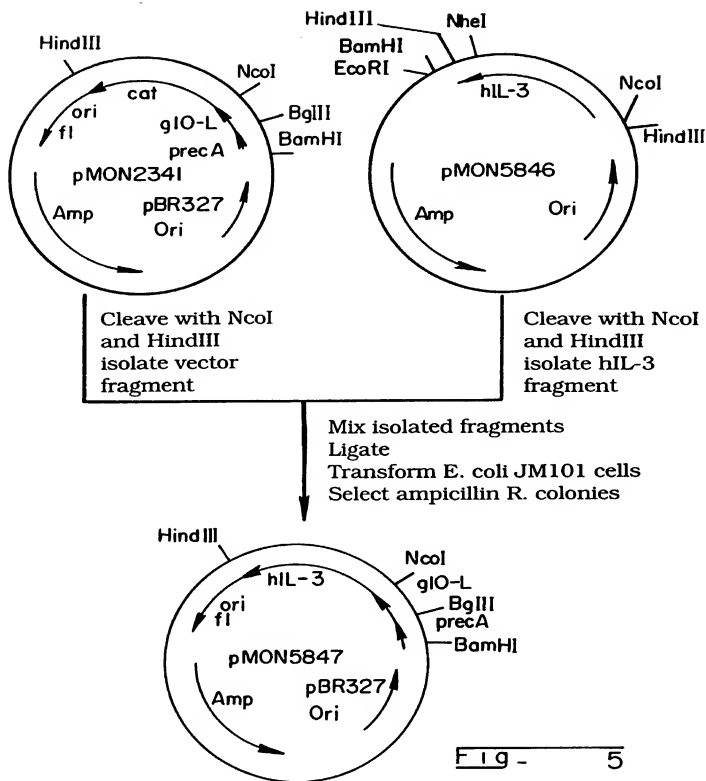
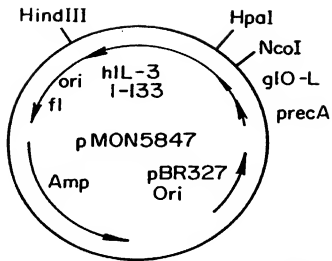


Fig - 5



Cleave with NcoI and HpaI.
 Klenow fill NcoI end to
 render it blunt.
 Ligate the Blunt ends.
 Transform E. coli JM101
 to ampicillin resistance

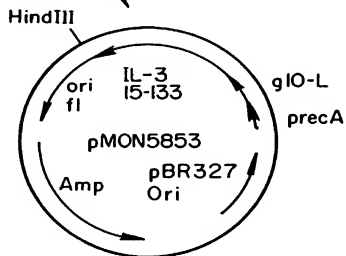
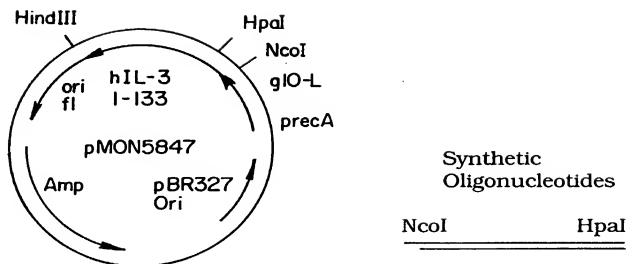


Fig - 6



Cleave with NcoI and HpaI.

Mix cleaved plasmid
with oligonucleotides.
Transform E. coli JM101
cells to ampicillin resistance.

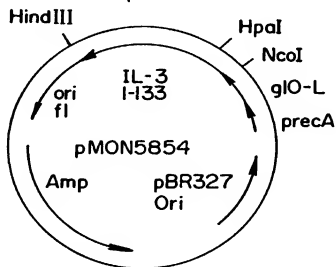


Fig - 7

1 ATGATGATTACTCTGCGCAAACTTCCTCTGGCGGTTGCCGTGCGCAGCGGGCGTAATGTCT 60
 TACTACTAATGAGACGCGTTTGAAGGAGACCGCCAACGGCAGCGTCGCCCGCATACAGA
 MetMetIleThrLeuArgLysLeuProLeuAlaValAlaValAlaAlaGlyValMetSer

N
 C
 O
 I
 GCTCAGGCCATGGCTAACTGC [SEQ ID NO: 149]
 61 -----+-----+-- 81
 CGAGTCCGGTACCGATTGACG [SEQ ID NO: 150]
 AlaGlnAlaMetAlaAsnCys [SEQ ID NO: 14]

lamB Signal Peptide

FIG- 8

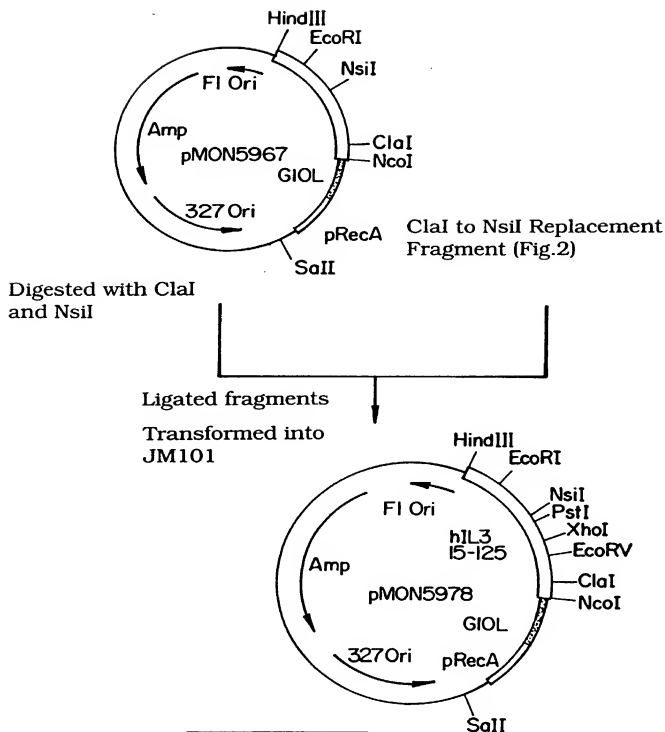
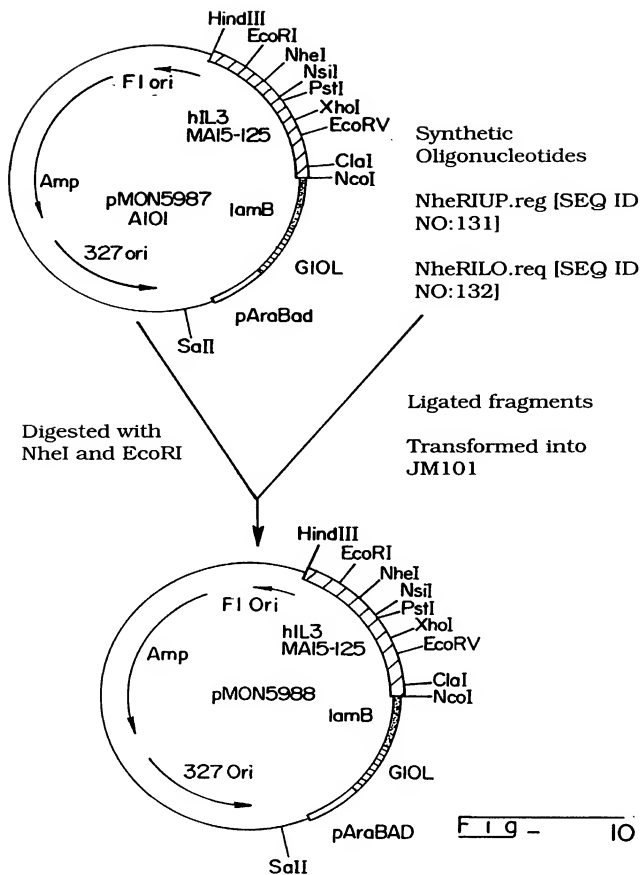
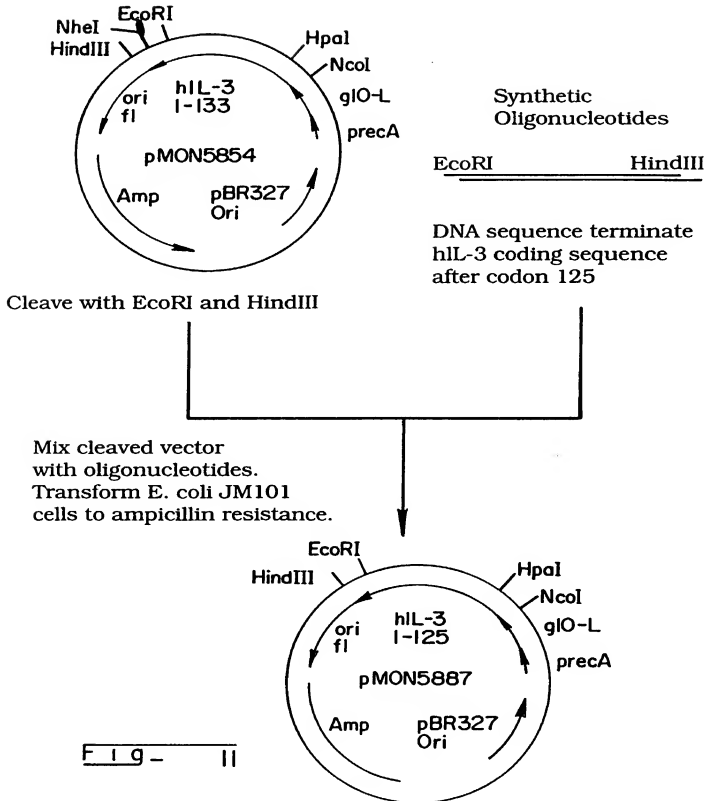


Fig - 9





5' CATGGCTAACTGCTCTAACATGAT 3'
SEQ ID NO:151

3' CGATTGACGAGATTGTACTAGC 5'
SEQ ID NO:152

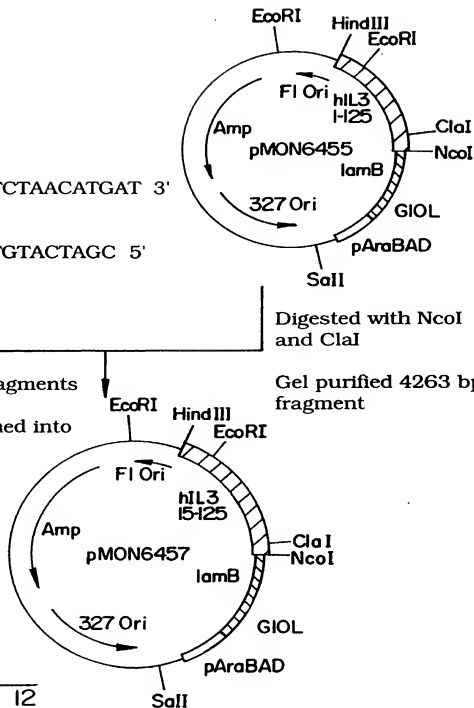
Annealed
Oligonucleotides

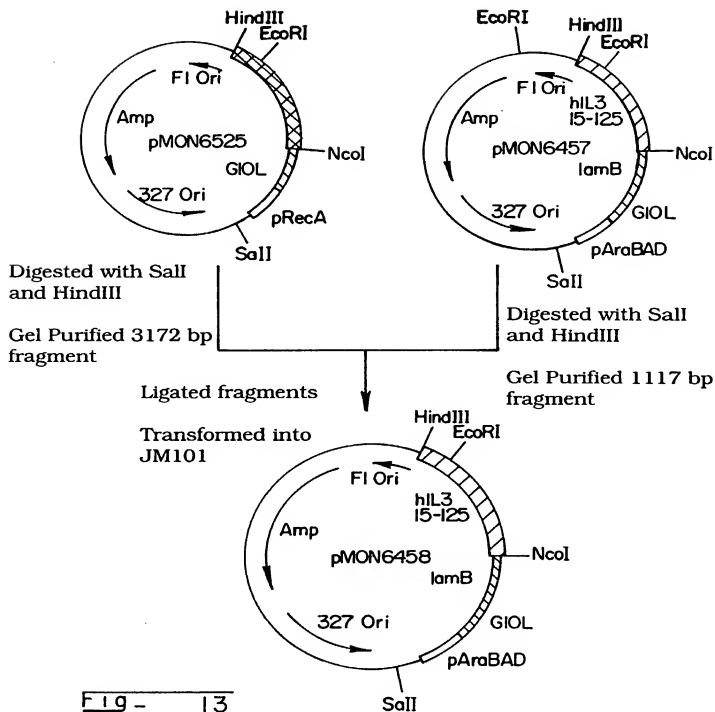
Digested with NcoI
and ClaI

Ligated fragments

Gel purified 4263 bp
fragment

Transformed into
JM101





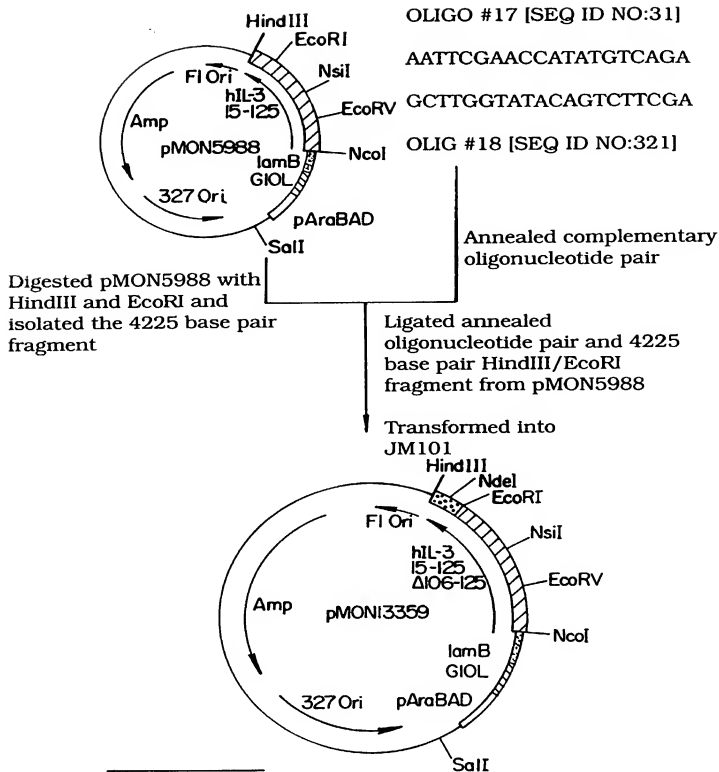
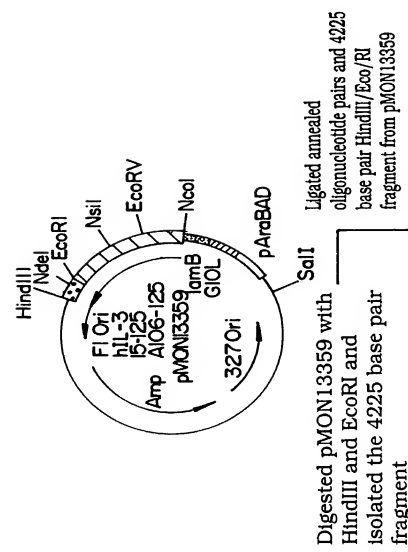
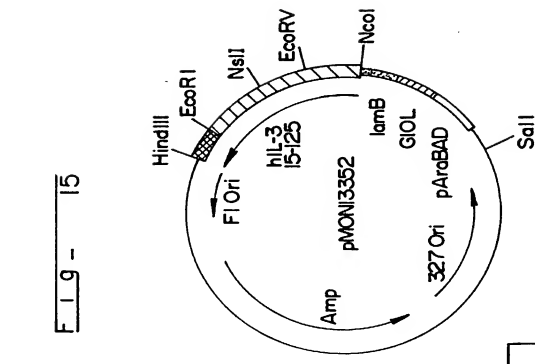


FIG - 14

Fig - 15



Digested pMON13359 with HindIII and EcoRI and isolated the 4225 base pair fragment

Ligated annealed oligonucleotide pairs and 4225 base pair HindIII/EcoRI fragment from pMON13359

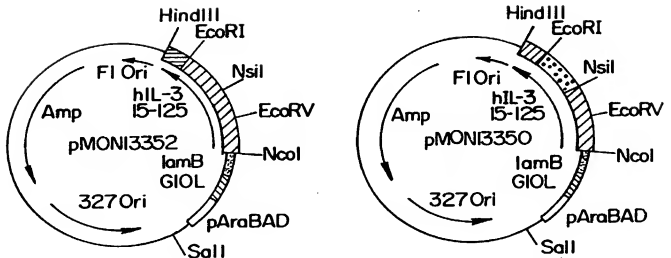
Transformed into JM101

Annealed complementary oligonucleotide pairs

OLIGO #45 [SEQ ID NO:59]
 5' AATCCCGGAAAAAAGTACGTTCTATCTGGTT 3'
 3' GGCCCTTTTGACTGCAAGATAGACCAAGGGAACCTCG 5'
 OLIGO #46 [SEQ ID NO:60]

OLIGO #49 [SEQ ID NO:63]
 5' TCCCTTGAGCAAGCGGAGGAACACAGTAATA 3'
 3' TTCGCGTCCCTGTGTGTCATTATCGA 5'
 OLIGO #50 [SEQ ID NO:64]

Fig - 16



Digested pMONI3352 with
NsiI and EcoRI and
isolated the 4178 base pair
fragment

Digested pMONI3350 with
NsiI and EcoRI and
isolated the 111 base pair
fragment

Ligated fragments

Transformed into
JM101

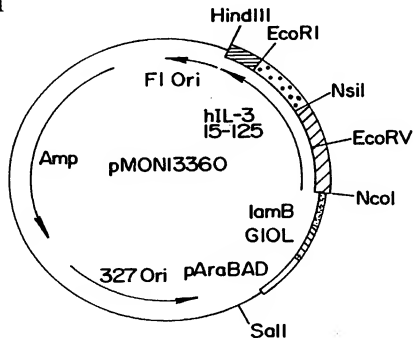
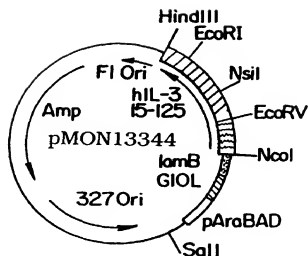
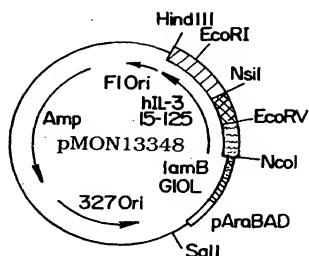


Fig - 17



Digested pMON13344 with NsiI and EcoRV and isolated the 4218 base pair fragment



Digested pMON13348 with NsiI and EcoRV and isolated the 71 base pair fragment

Ligated fragments

Transformed into JM101

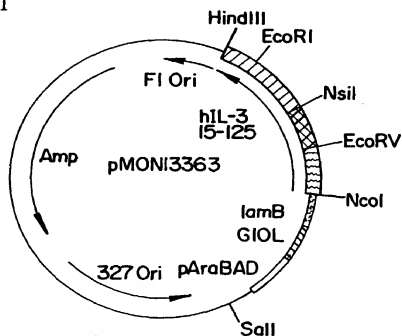
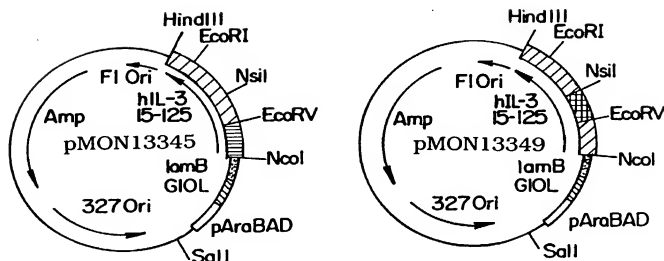


Fig - 18



Digested pMON13345 with
NsiI and EcoRV and
isolated the 4218 base pair
fragment

Digested pMON13349 with
NsiI and EcoRV and
isolated the 71 base pair
fragment

Ligated fragments

Transformed into
JM101

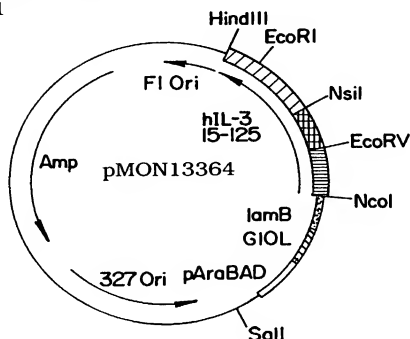


Fig - 19

